



2001

## Determination of flavonoids in *Daphnis Genkwae* Flos by high performance liquid chromatography

Follow this and additional works at: <https://www.jfda-online.com/journal>

---

### Recommended Citation

Lin, J.-H.; Lin, Y.-T.; Chiou, Y.-N.; Wen, K.-C.; and Liao, C.-H. (2001) "Determination of flavonoids in *Daphnis Genkwae* Flos by high performance liquid chromatography," *Journal of Food and Drug Analysis*: Vol. 9 : Iss. 1 , Article 8.

Available at: <https://doi.org/10.38212/2224-6614.2811>

This Original Article is brought to you for free and open access by Journal of Food and Drug Analysis. It has been accepted for inclusion in Journal of Food and Drug Analysis by an authorized editor of Journal of Food and Drug Analysis.

# Determination of Flavonoids in *Daphnis Genkwae* Flos by High Performance Liquid Chromatography

JER-HUEI LIN, YA-TZE LIN, YI-NING CHIOU, KUO-CHING WEN\* AND CHUN-HENG LIAO

National Laboratories of Foods and Drugs, Department of Health, Executive Yuan 161-2, Kuen-Yang Street, Nankang, 11513, Taipei, Taiwan

(Received: August 21, 2000; Accepted: November 22, 2000)

## ABSTRACT

In order to evaluate the quality of *Daphnis Genkwae* Flos, a high performance liquid chromatographic method (HPLC) was developed. Six flavonoid constituents from *Daphnis Genkwae* Flos, genkwainin 5-*O*- $\beta$ -D-primeveroside (**1**), genkwainin 5-*O*- $\beta$ -D-glucoside (**2**), genkwainin (**3**), potassium apigenin 7-*O*- $\beta$ -D-glucuronate (**4**), apigenin (**5**) and tiliroside (**6**) determined by this simple, rapid and accurate method.

Samples were analyzed using a Cosmosil 5C<sub>18</sub>-AR reversed phase column by gradient elution with varied proportion of 1.0 % (v/v) acetic acid and acetonitrile as mobile phase at 254 nm. Sulfamethoxypridazine was used as the internal standard.

Regression equations revealed the linear relationship (correlation coefficients: 0.9994~0.9999) between the peak-area ratios flavonoid to internal standard and concentrations of flavonoids. The recoveries of six flavonoids were between 100.9~106.1%. The relative standard deviations of six flavonoids ranged between 0.49~3.46% (intraday) and 0.52~4.61% (interday). The contents of the six flavonoids in sixteen crude drugs of *Daphnis Genkwae* Flos were **1** 0.13~0.40%, **2** 0.02~0.12%, **3** 0.24~0.56%, **4** 0.16~1.19%, **5** 0.30~0.73% and **6** 0.15~0.57%, respectively.

Key words: *Daphnis Genkwae* Flos, flavonoids, HPLC

## INTRODUCTION

*Daphnis Genkwae* Flos is the dried flower of *Daphne genkwa* Sieb et Zucc. (Thymelaeaceae) which inhibits the growth of bacteria and promotes water excretion from the body and is used as a diuretic<sup>(1)</sup>. Pharmacological examination has showed that it inhibits adenosine 3', 5'-cyclic monophosphate phosphodiesterase and xanthine oxidase<sup>(2~3)</sup>. The market volume of traditional Chinese medicines has been increasing in Taiwan, therefore, the quality control for traditional Chinese medicines is important.

In a previous report, we isolated twelve flavonoids from *Daphnis Genkwae* Flos<sup>(4)</sup>. A literature search indicated that some of these flavonoids possessed pharmacological activities<sup>(3, 5~6)</sup>, and could be used as markers for chemical evaluation. In this study, six flavonoids present in higher content among twelve flavonoids: genkwainin 5-*O*- $\beta$ -D-primeveroside (**1**), genkwainin 5-*O*- $\beta$ -D-glucoside (**2**), genkwainin (**3**), potassium apigenin 7-*O*- $\beta$ -D-glucuronate (**4**), apigenin (**5**) and tiliroside (**6**) were used as the marker constituents and their structures were shown in Figure 1.

High performance liquid chromatography (HPLC) has been extensively used for analysis and provides high resolution and reproducible results. Many traditional Chinese medicines have been analyzed by HPLC<sup>(7~8)</sup>. For the determination of these six flavonoids, a rapid and accurate reversed-phase HPLC method was developed. The contents of six constituents of *Daphnis Genkwae* Flos in sixteen samples from markets were also determined.

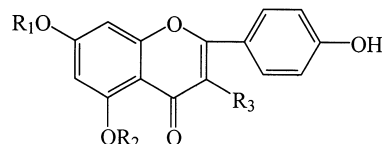
## MATERIALS AND METHODS

### I. Materials

Crude drugs of *Daphnis Genkwae* Flos were obtained from local dealers of Chinese herbs in Taiwan and their origins were verified by Dr. Hsien Chang Chang, a research fellow in our laboratory.

### II. Chemicals and Reagents

Reference standards, genkwainin 5-*O*- $\beta$ -D-primeveroside, genkwainin 5-*O*- $\beta$ -D-glucoside, genkwainin, potassium apigenin 7-*O*- $\beta$ -D-glucuronate, apigenin, and tiliroside were isolated from the flowers of *Daphne genkwa*. Sulfamethoxypridazine was purchased from Sigma (St. Louis, MO),



	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
<b>1</b>	CH <sub>3</sub>	primeverose	H
<b>2</b>	CH <sub>3</sub>	glucose	H
<b>3</b>	CH <sub>3</sub>	H	H
<b>4</b>	K glucuronate	H	H
<b>5</b>	H	H	H
<b>6</b>	H	H	-O-courmaroyl-glucose

Figure 1. Structures of six flavonoids **1**~**6** from *Daphnis Genkwae* Flos.

\* Author for correspondence. Tel: 02-26531208; Fax: 02-26531213; E-mail:kuochingwen@nlf.gov.tw

U.S.A.). Acetic acid was purchased from Nacalai (Tokyo, Japan). Acetonitrile, methanol and acetone (HPLC grade) were purchased from BDH (Poole, England). Ultrapure distilled water with a resistance greater than 18 M $\Omega$  was used.

### III. Instruments and Conditions

The analysis was performed using a Waters 600E HPLC pump with a Waters 486 tunable absorbance detector and a Shimadzu SIL-9A auto-injector. A Cosmosil 5C<sub>18</sub>-AR (5 $\mu$ m, 4.6 mm I.D. $\times$ 250 mm) was used. Peak areas were calculated with a Shiunn Haw computing integrator.

The mobile phase was the mixtures of 1.0 % (v/v) acetic acid (A) and acetonitrile (B) with a gradient elution. The condition was shown as follows:

Time (min)	A	B	
0	90	10	*
5	80	20	Linear
18	80	20	Linear
40	0	100	Linear

The flow rate was 1.0 mL/min and the detecting wavelength was 254 nm. The concentration of internal standard was 16.7  $\mu$ g/mL.

### IV. Preparation of Standard Solutions

Accurate amount of genkwanin 5-*O*- $\beta$ -D-primeveroside, genkwanin 5-*O*- $\beta$ -D-glucoside, genkwanin, potassium apigenin 7-*O*- $\beta$ -D-glucuronate, apigenin and tiliroside were dissolved in methanol then an appropriate amount of internal standard solution was added to give various concentrations of flavonoids. The concentration ranges of **1**~**6** were as followed: **1** 5~80  $\mu$ g/mL, **2** 1~16  $\mu$ g/mL, **3** 5~80  $\mu$ g/mL, **4** 10.2~95.2  $\mu$ g/mL, **5** 6~96  $\mu$ g/mL and **6** 6~96  $\mu$ g/mL. Calibration curves were plotted based on linear regression analysis of peak-areas versus concentrations.

### V. Extraction Conditions

In order to obtain better extraction of flavonoids from crude drug, solvents such as acetone, methanol and 70 % methanol, have been tried. The solvents and conditions for extraction were described as follows and named as **A**, **B**, **C**, **D** and **E**.

- A:** 30 mL of acetone one time, and then 20 mL five times.
  - B:** 30 mL of methanol one time, and then 20 mL five times.
  - C:** 30 mL of 70 % methanol one time, and then 20 mL five times.
  - D:** 30 mL of methanol one time, 20 mL two times, and then with 20 mL of 70 % methanol three times.
  - E:** 30 mL of acetone one time, 20 mL two times, and then with 20 mL of 70 % methanol three times.
- All samples were cut into pieces and refluxed with **A**~**E**

at 80°C for one hour. The extracts were filtered while hot and then diluted. A suitable amount of internal standard was added to the solution to give a concentration of 16.7  $\mu$ g/mL.

All extracts were filtered through 0.45  $\mu$ m Millipore filters and the extraction ratios of flavonoids were then calculated.

### VI. Preparation of Sample Solution

All crude drugs were cut into pieces and dried at 55°C for 24 hours. A sample (1.0g) was accurately weighed and extracted three times with methanol (30, 20 and 20 mL, successively) and two times with 70% methanol (20 and 20 mL, successively) at 80°C for one hour. The extracts were combined and filtered into a volumetric flask, a suitable amount of internal standard was then added to the solution to give a final concentration of 16.7  $\mu$ g/mL, and methanol was added to 150 mL. This solution was filtered through a 0.45  $\mu$ m filter before use.

### VII. Solution for Recovery Studies

Different amounts of genkwanin 5-*O*- $\beta$ -D-primeveroside, genkwanin 5-*O*- $\beta$ -D-glucoside, genkwanin, potassium apigenin 7-*O*- $\beta$ -D-glucuronate, apigenin and tiliroside were added to a sample solution of Daphnis Genkwae Flos which the content were measured and a suitable amount of internal standard was added to give a final concentration of 16.7  $\mu$ g/mL. All sample solutions were filtered, subjected to HPLC analysis, and the concentrations of six flavonoids were calculated from their calibration graphs.

## RESULTS AND DISCUSSION

### I. Analytical Conditions

All six flavonoids and sulfamethoxy pyridazine were successfully determined in a single run HPLC. By using gradient elution, genkwanin 5-*O*- $\beta$ -D-primeveroside, potassium apigenin 7-*O*- $\beta$ -D-glucuronate, genkwanin 5-*O*- $\beta$ -D-glucoside, tiliroside, apigenin, genkwanin and sulfamethoxy pyridazine were resolved and eluted at 23.6 min, 26.7 min, 28.2 min, 32.0 min, 34.4 min, 38.5 min and 15.9 min, respectively (Figure 2).

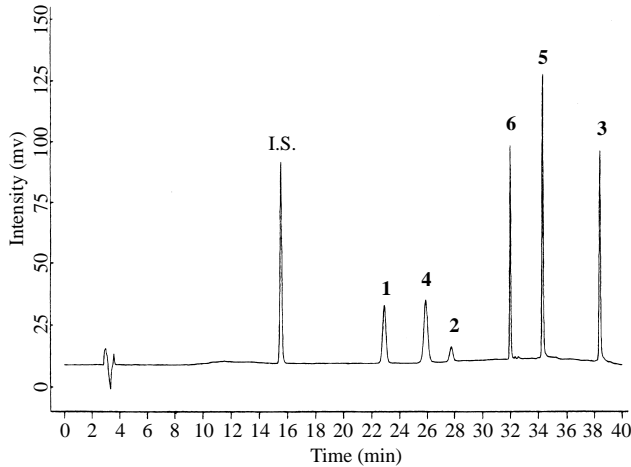
### II. Calibration Graphs for Six Flavonoids

The linearity of the plot of peak area ratio (X) vs. concentration (Y,  $\mu$ g/mL) for each of the flavonoid was investigated. The regression (Table 1) revealed linear relationships (correlation coefficients: 0.9994~0.9999) between the peak-area ratios flavonoid to internal standard and concentrations.

### III. System Suitability Test

To assess the precision of these methods, standard solutions of genkwanin 5-*O*- $\beta$ -D-primeveroside, genkwanin 5-*O*-

$\beta$ -D-glucoside, genkwanin, potassium apigenin 7-O- $\beta$ -D-glucuronate, apigenin and tiliroside were determined six times on the same day and a six day period. The intraday and interday variation studies, indicated that the relative standard deviations were less than 3.46 and 4.61%, respectively (Table



**Figure 2.** HPLC chromatogram of genkwanin 5-O- $\beta$ -D-primeveroside (1), genkwanin 5-O- $\beta$ -D-glucoside (2), genkwanin (3), potassium apigenin 7-O- $\beta$ -D-glucuronate (4), apigenin (5) and tiliroside (6). I.S. for internal standard (sulfamethoxypyridazine).

**Table 1.** Regression equations and their correlation coefficients ( $r$ ) of six flavonoids

Constituent <sup>a</sup>	Linear range ( $\mu\text{g/mL}$ )	Linear equations	$r$
1	5~80	$Y = 39.17X - 0.21$	0.9999
2	1~16	$Y = 27.78X - 0.03$	0.9994
3	5~80	$Y = 23.59X + 0.66$	0.9999
4	10.2~95.2	$Y = 38.18X + 0.65$	0.9999
5	6~96	$Y = 23.39X + 0.43$	0.9999
6	6~96	$Y = 34.74X + 0.15$	0.9999

<sup>a</sup> Constituent 1: genkwanin 5-O- $\beta$ -D-primeveroside, 2: genkwanin 5-O- $\beta$ -D-glucoside, 3: genkwanin, 4: potassium apigenin 7-O- $\beta$ -D-glucuronate, 5: apigenin and 6: tiliroside.

2). The results for the recoveries of six flavonoids ranged from 100.9 to 106.1% (Table 3). The method proved to be precise and accurate.

#### IV. Extraction Conditions

In order to obtain a good extraction of flavonoids from crude drugs, five kinds of solvents were tested (A~E). The results (Table 4) indicated that the condition D (30 mL of methanol one time, 20 mL two times, and then 20 mL of 70% methanol three times at 80°C for one hour) afforded the highest yield of the six flavonoids.

#### V. Determination of Six Flavonoids in *Daphnis Gankwae Flos*

**Table 2.** Intraday and interday analytical precisions of six flavonoids of *Daphnis Gankwae Flos* (n=6)

Constituent <sup>a</sup>	Concentration ( $\mu\text{g/mL}$ )	Intraday R.S.D. (%)	Interday R.S.D. (%)
1	5.0	3.27	3.22
	20.0	1.31	0.95
	80.0	0.81	0.52
2	1.0	3.46	4.61
	4.0	1.49	1.91
	16.0	1.39	2.91
3	5.0	2.99	2.38
	20.0	1.06	3.56
	80.0	0.93	1.24
4	10.2	1.50	2.87
	27.2	1.20	1.87
	95.2	1.00	1.86
5	6.0	0.97	1.73
	24.0	0.49	2.02
	96.0	2.21	1.65
6	6.0	0.99	2.53
	24.0	1.37	2.99
	96.0	1.26	1.32

<sup>a</sup> Constituents 1~6 are the same as Table 1.

**Table 3.** Recoveries of six flavonoids from extract of *Daphnis Genkwae Flos*

Constituent <sup>a</sup>	Amount added ( $\mu\text{g/mL}$ )	Amount measured ( $\mu\text{g/mL}$ )	Recovery (%)	Mean $\pm$ S.D. <sup>b</sup> (%)	R.S.D. (%)
1	10.0	10.7	107.1	106.1 $\pm$ 0.79	0.74
	20.0	21.1	105.2		
	40.0	42.4	105.9		
2	1.3	1.3	103.1	105.0 $\pm$ 1.89	1.80
	2.5	2.6	104.2		
	3.8	4.0	107.6		
3	5.0	5.1	101.2	100.9 $\pm$ 0.25	0.25
	10.0	10.1	100.6		
	20.0	20.2	100.8		
4	10.2	10.6	103.8	105.4 $\pm$ 1.13	1.07
	20.4	21.6	105.9		
	30.6	32.6	106.4		
5	15.0	15.6	104.0	103.9 $\pm$ 0.82	0.79
	30.0	31.4	104.8		
	45.0	46.3	102.8		
6	15.0	15.7	104.4	105.2 $\pm$ 0.67	0.63
	30.0	31.8	106.0		
	45.0	47.3	105.2		

<sup>a</sup> Constituents 1~6 are the same as Table 1.

<sup>b</sup> Sample size n=3.

**Table 4.** The relative extraction ratio of six flavonoids from *Daphnis Genkwae Flos*

Solvent	Constituent <sup>a</sup>					
	1 (%)	2 (%)	3 (%)	4 (%)	5 (%)	6 (%)
A	7.9	7.2	60.7	0.0	71.6	3.3
B	81.4	86.1	98.9	88.8	92.7	88.7
C	84.7	95.2	88.9	95.0	88.6	94.4
D	100.0	100.0	100.0	100.0	100.0	100.0
E	72.6	76.7	87.4	83.6	87.8	86.3

<sup>a</sup> Constituents 1~6 are the same as Table 1.

A: 30 mL of acetone one time, and then 20 mL five times.

B: 30 mL of methanol one time, and then 20 mL five times.

C: 30 mL of 70% methanol one time, and then 20 mL five times.

D: 30 mL of methanol one time, 20 mL two times, and then with 20 mL of 70% methanol three times.

E: 30 mL of acetone one time, 20 mL two times, and then with 20 mL of 70% methanol three times.

**Table 5.** The content (Mean  $\pm$  S.D.<sup>b</sup>, %) of six flavonoids in 16 crude drugs of *Daphnis Genkwae Flos*

No of sample	Constituent <sup>a</sup>					
	1	2	3	4	5	6
1	0.16 $\pm$ 0.007	0.06 $\pm$ 0.001	0.32 $\pm$ 0.005	0.68 $\pm$ 0.009	0.32 $\pm$ 0.006	0.22 $\pm$ 0.003
2	0.14 $\pm$ 0.010	0.05 $\pm$ 0.002	0.31 $\pm$ 0.010	0.68 $\pm$ 0.002	0.32 $\pm$ 0.004	0.19 $\pm$ 0.003
3	0.15 $\pm$ 0.007	0.08 $\pm$ 0.002	0.36 $\pm$ 0.013	0.80 $\pm$ 0.015	0.38 $\pm$ 0.013	0.57 $\pm$ 0.004
4	0.30 $\pm$ 0.013	0.10 $\pm$ 0.004	0.42 $\pm$ 0.012	1.08 $\pm$ 0.022	0.73 $\pm$ 0.020	0.20 $\pm$ 0.002
5	0.27 $\pm$ 0.009	0.08 $\pm$ 0.008	0.36 $\pm$ 0.017	1.19 $\pm$ 0.004	0.51 $\pm$ 0.018	0.27 $\pm$ 0.001
6	0.13 $\pm$ 0.004	0.05 $\pm$ 0.007	0.37 $\pm$ 0.010	0.46 $\pm$ 0.007	0.50 $\pm$ 0.024	0.19 $\pm$ 0.001
7	0.15 $\pm$ 0.006	0.02 $\pm$ 0.001	0.24 $\pm$ 0.004	0.75 $\pm$ 0.014	0.63 $\pm$ 0.018	0.18 $\pm$ 0.002
8	0.40 $\pm$ 0.003	0.08 $\pm$ 0.004	0.56 $\pm$ 0.005	0.43 $\pm$ 0.006	0.60 $\pm$ 0.004	0.22 $\pm$ 0.001
9	0.20 $\pm$ 0.016	0.06 $\pm$ 0.005	0.34 $\pm$ 0.009	0.51 $\pm$ 0.007	0.61 $\pm$ 0.010	0.16 $\pm$ 0.002
10	0.30 $\pm$ 0.004	0.12 $\pm$ 0.011	0.43 $\pm$ 0.018	1.11 $\pm$ 0.020	0.61 $\pm$ 0.021	0.21 $\pm$ 0.004
11	0.24 $\pm$ 0.007	0.08 $\pm$ 0.005	0.44 $\pm$ 0.015	0.16 $\pm$ 0.004	0.35 $\pm$ 0.015	0.15 $\pm$ 0.001
12	0.26 $\pm$ 0.002	0.09 $\pm$ 0.004	0.33 $\pm$ 0.009	0.97 $\pm$ 0.014	0.50 $\pm$ 0.010	0.20 $\pm$ 0.004
13	0.20 $\pm$ 0.003	0.07 $\pm$ 0.003	0.41 $\pm$ 0.002	0.82 $\pm$ 0.004	0.60 $\pm$ 0.006	0.22 $\pm$ 0.002
14	0.14 $\pm$ 0.004	0.07 $\pm$ 0.002	0.33 $\pm$ 0.006	0.75 $\pm$ 0.006	0.31 $\pm$ 0.009	0.21 $\pm$ 0.001
15	0.27 $\pm$ 0.005	0.09 $\pm$ 0.002	0.36 $\pm$ 0.003	0.91 $\pm$ 0.008	0.38 $\pm$ 0.004	0.27 $\pm$ 0.001
16	0.15 $\pm$ 0.005	0.05 $\pm$ 0.004	0.32 $\pm$ 0.001	0.66 $\pm$ 0.002	0.30 $\pm$ 0.003	0.20 $\pm$ 0.002
Range	0.13 ~ 0.40	0.02 ~ 0.12	0.24 ~ 0.56	0.16 ~ 1.19	0.30 ~ 0.73	0.15 ~ 0.57
Mean $\pm$ S.D.	0.22 $\pm$ 0.077	0.071 $\pm$ 0.023	0.37 $\pm$ 0.071	0.75 $\pm$ 0.274	0.48 $\pm$ 0.141	0.23 $\pm$ 0.951

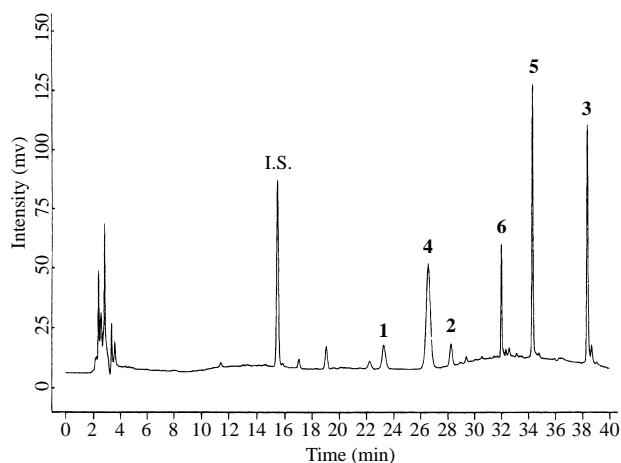
<sup>a</sup> Constituents 1~6 are the same as Table 1.

<sup>b</sup> Sample size n=3.

The sample solution was analyzed by HPLC and the chromatogram is shown in Figure 3. The peaks were identified by comparison of the retention time and UV spectra with those of authentic samples. The chromatogram showed no interference with the six flavonoids. The contents of the six flavonoids in 16 crude drugs were successfully determined within forty minutes and calculated as shown in Table 5. The contents of the individual flavonoids in the *Daphnis Genkwae Flos* were **1**, 0.13~0.40%; **2**, 0.02~0.12%; **3**, 0.24~0.56%; **4**, 0.16~1.19%; **5**, 0.30~0.73%; and **6**, 0.15~0.57%, respectively. The contents varied from sample to sample with the largest difference 7.5-folds for potassium apigenin 7-*O*- $\beta$ -D-glucuronate and the smallest difference for genkwainin 2.4-folds.

From the above results, it can be concluded that this method for the determination of the flavonoids in *Daphnis Genkwae Flos* is suitable. How to produce *Daphnis Genkwae Flos* with consistent contents of flavonoids requires further study.

## ACKNOWLEDGEMENTS



**Figure 3.** HPLC chromatogram of methanol extract of *Daphnis Genkwae Flos*. Peak identity: same as Figure 2.

Financial support came from the National Health Research Institute, Republic of China (DOH-86-HR-607) special thanks to Dr. Hsien Chang Chang for his kind identification of plant materials.

## REFERENCES

1. Chang, H. M. and But, P. H. 1986. Pharmacology and Applications of Chinese Materia Medica. Vol. I. pp. 567~574. World Scientific Publishing, Singapore.
2. Nikaido, T., Ohmoto, T. and Sankawa, U. 1987. Inhibitors of adenosine 3', 5'-cyclic monophosphate phosphodiesterase in *Daphne genkwa* SIEB. *Et ZUCC.. Chem. Pharm. Bull.* 35: 675-681.
3. Noro, T., Oda, Y., Miyase, T., Ueno, A. and Fukushima, S. 1983. Inhibitors of xanthine oxidase from flowers and buds of *Daphne genkwa*. *Chem. Pharm. Bull.* 31: 3984-3987.
4. Lin, J. H., Lin, Y. T., Huang, Y. J., Wen, K. C., Chen, R. M., Ueng, T. H. and Lao, C. H. 2000. Isolation and cytotoxicity of the flavonoids from *Daphnis genkwae* Flos. *Journal of Food and Drug Analysis* 9: 6-11.
5. Okamura, N., Haraguchi, H., Hashimoto, K. and Yagi, A. 1994. Flavonoids in *Rosmarinus officinalis* leaves. *Phytochemistry* 37: 1463-1466.
6. Perry, N. B. and Foster, L. M. 1994. Antiviral and anti-fungal flavonoids, plus a triterpene, from *Hebe cupressoides*. *Planta Med.* 60: 491-492.
7. Ku, Y. R., Lin, Y. T., Wen, K. C., Lin, J. H. and Lao, C. H. 1996. Determination of parishin, parishins B and C in traditional Chinese medicinal preparations by high performance liquid chromatography. *Journal of Liquid Chromatography & Related Technologies* 19: 3265-3277.
8. Lin, J. H., Ku, Y. R., Huang, Y. S., Wen, K. C. and Lao, C. H. 1997. Determination of polar constituents in traditional Chinese medicinal prescriptions by high performance liquid chromatography. *Journal of Liquid Chromatography & Related Technologies* 20: 1617-1632.

## 芫花藥材中類黃酮成分之高效液相層析定量研究

林哲輝 林雅姿 邱怡寧 溫國慶\* 廖俊亨

行政院衛生署藥物食品檢驗局  
台北市南港區昆陽街161-2號

(收稿：August 21, 2000；接受：November 22, 2000)

### 摘 要

本研開發用高效液相層析法，分析芫花藥材中之六種類黃酮成分 genkwanin 5-*O*- $\beta$ -D-primeveroside (1), genkwanin 5-*O*- $\beta$ -D-glucoside (2), genkwanin (3), potassium apigenin 7-*O*- $\beta$ -D-glucuronate (4), apigenin (5) 及 tiliroside (6)，並進一步以此高效液相層析法調查市售芫花藥材中此六種成分之含量。

本實驗之高效液相層析法係採用 C<sub>18</sub> 逆向層析管柱，移動相為乙腈-1.0% 醋酸溶液，利用線性梯度沖提，檢測波長為 254 nm，得到良好分析結果。其線性迴歸方程式及相關係數(r)分別為：1：Y = 39.17X - 0.21 (r = 0.9999)；2：Y = 27.78X - 0.03 (r = 0.9994)；3：Y = 23.59X + 0.66 (r = 0.9999)；4：Y = 38.18X + 0.65 (r = 0.9999)；5：Y = 23.39X + 0.43 (r = 0.9999)；6：Y = 34.74X + 0.15 (r = 0.9999)，均呈良好線性關係。此六種類黃酮成分之添加回收率試驗結果為 100.9%~106.1%；同日內及異日間相對標準偏差試驗，其同日內為 0.49~3.46%，異日間為 0.52~4.61%，顯示此高效液相層析法用於芫花藥材之分析其效果良好。

市售芫花藥材 16 種，利用上述高效液相層析法，分析此六種類黃酮成分的含量，結果 1~6 的含量分別如下：1：0.13~0.40%；2：0.02~0.12%；3：0.24~0.56%；4：0.16~1.19%；5：0.30~0.73%；6：0.15~0.57%。

關鍵詞：芫花，類黃酮，高效液相層析